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Compensatory renal growth in uninephrectomized adult mice is growth hormone dependent

Allan Flyvbjerg, William F. Bennett, Ruth Rasch, Johan W. van Neck, Cora A.H. Groffen, John J. Kopchick, and John A. Scarlett

Medical Department M, Medical Research Laboratory M, Institute of Experimental Clinical Research, Aarhus Kommunehospital, Aarhus C, Denmark; Sensus Drug Development Corporation, Austin, Texas, USA; Department of Cell Biology, Institute of Anatomy, University of Aarhus, Aarhus, Denmark; Laboratory for Pediatrics, Erasmus University Rotterdam, Rotterdam, The Netherlands; and Edison Biotechnology Institute, Department of Biomedical Sciences, Ohio University, Athens, Ohio, USA

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Background. Growth hormone (GH) and insulin-like growth factors (IGFs) have been implicated as pathogenic factors in compensatory renal growth (CRG) following unilateral nephrectomy in rodents. CRG in adult rats has been suggested to be GH dependent and GH independent in immature rats. However, the exact role of GH as a regulating or permissive factor in CRG in adult rodents has not been fully resolved to date.

Methods. To elucidate a possible direct, permissive role of GH in CRG, we examined the effect of a newly developed specific GH receptor (GHR) antagonist (G120K-PEG) on kidney IGF-I accumulation and renal/glomerular hypertrophy over seven days after uninephrectomy in adult mice.

Results. Placebo-treated uninephrectomized mice were characterized by a transient increase in kidney IGF-I concentration preceding CRG and an increase in glomerular volume. In G120K-PEG–treated uninephrectomized animals, increased kidney IGF-I levels, kidney weight, and glomerular volume were fully abolished. No differences were seen between the two uninephrectomized groups with respect to body weight, food intake, blood glucose, serum GH, IGF-I, or IGFBP-3 levels.

Conclusions. The administration of a GHR antagonist in uninephrectomized adult mice has renal effects without affecting circulating levels of GH/IGFs, indicating that the effect of G120K-PEG may be mediated through a direct inhibitory effect on renal IGF-I accumulation through the renal GHR. This study shows, to our knowledge for the first time, that CRG in adult mice is strictly GH dependent.

Growth hormone (GH) and insulin-like growth factors (IGFs) have been implicated as pathogenic factors in compensatory renal growth (CRG) following unilateral nephrectomy [1–10]. The first published indirect evidence that the GH/IGF axis may be involved in CRG arose from studies in which a blunted CRG response was seen in unilaterally nephrectomized hypophysectomized rats [1, 2]. In addition, adults rats exposed to unilateral nephrectomy have been associated with an early increase in pulsatile GH release [10], and furthermore, the administration of a long-acting somatostatin analogue (octreotide) [6] or a GH-releasing factor antagonist [9] has been shown to abolish the early CRG. Several studies have shown an early rise in extractable or immunoassayable kidney IGF-I content in the remnant kidney following unilateral nephrectomy in adult rats [3, 5–7]. However, the mechanism responsible for this rise in renal IGF-I is still a matter of debate, as different changes in gene expression of the IGF axis have been reported in adult and immature rats during CRG [7–11]. Finally, in adult rats, CRG has been shown to be GH dependent, whereas the process in immature rats is GH independent [8–11]. Based on these studies, it seems evident that CRG, both in immature and adult rodents, is IGF dependent; However, thus far the precise role of GH in adult rodents as a modulating or strictly permissive factor for CRG has not yet been resolved. Recently, a series of highly specific GH receptor (GHR) antagonists has been developed for potential therapeutic use [12–18]. Accordingly, the aim of this study was to examine the effect of a specific GHR antagonist (G120K-PEG) on the kidney GH/IGF-I system, renal enlargement, and glomerular hypertrophy in adult mice exposed to unilateral nephrectomy.

Key words: growth hormone, antagonist, nephrectomy, insulin-like growth factors, mRNA.

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METHODS

Animals and protocol

Adult female Balb/C(a) mice (Bomholtgaard, Ry, Denmark) with initial body weights of 15 to 19 g were used.

The mice were housed four per cage in a room with a 12:12 hour (7:00 a.m. to 7:00 p.m.) artificial light cycle, a temperature of 21 ± 2°C, and a humidity of 55 ± 2%. The animals had free access to standard chow (Altromin #1324; Lage, Germany) and tap water throughout the experiment. The animals were randomized into four groups, and two of these groups were exposed to a right-side nephrectomy for placebo (NP) or GHR antagonist (NA) treatment. One group was exposed to sham operation (S) by a flank incision with manipulation of the kidney, whereas the fourth group served as a nonoperated control group (C). All operations were performed under anesthesia with avertin (240 mg/kg body wt i.p.). Half of the animals exposed to unilateral nephrectomy were treated with subcutaneous injections of a pegylated GHR antagonist (G120K-PEG; Sensus, Austin, Texas, USA) [12–20]. The treatment was started immediately after unilateral nephrectomy and was given in a dose of 2 mg/kg body wt every second day to maintain high diurnal levels of the GHR antagonist. The other half of uninephrectomized mice were injected with an equivalent volume of vehicle (0.154 M NaCl). At days 0, 2, and 7 during the study, the following parameters were recorded: body weight, blood glucose, and fodder consumption. When animals were taken out for examination at days 0 (group C), 2 (groups S, NP, and NA), and 7 (groups S, NP, and NA; $N = 6$ in each group at each time point), anesthesia was performed with sodium barbital (10 mg/kg body wt i.p.), and blood was drawn exactly five minutes later from the retro-orbital venous plexus for determination of serum GH, IGF-I, and IGFBP-3. Serum was stored at −80°C until measurements were performed. Furthermore, the left kidneys were rapidly removed and carefully cleaned and weighed. A two millimeter thick horizontally cut slice from the middle of the left kidney (including the papilla) was fixed in 4% paraformaldehyde for morphological measurements. Finally, the two remnant pieces of each left kidney were snap frozen in liquid nitrogen for later determination of GHR, GH binding protein (GHBP), IGF-I gene-expression, and IGF-I peptide.

Immunoassays

Serum GH was measured by radioimmunoassay (RIA) using a specific polyclonal rabbit rat (r) GH antibody and rGH as the standard. Semilog linearity of mouse serum and rGH (in the standard) was found at multiple dilutions, which is an indication of antigen similarity between mouse GH and rGH. The ingredients, including ^{125}I-rGH, were obtained from Amersham International (Bucks, UK). Serum IGF-I was measured after extraction with acid ethanol. The mixture was incubated for two hours at room temperature and was centrifuged, and 25 μl of the supernatant were diluted 1:200 before analysis [18, 19]. Kidney extraction was performed as previously described [18, 19]. Briefly, 80 to 100 mg of tissue were homogenized on ice in 1 M acetic acid (5 ml/g tissue) with an Ultra Turrax TD 25 and were further disrupted using a Potter Elvehjelm homogenizer (both from Janke-Kunkel GmbH, Staufen, Germany). With this procedure, all IGFBPs were removed from kidney tissue [19]. After lyophilization, the samples were redissolved in phosphate buffer (pH 8.0) and kept at −80°C until the IGF-I assay was performed in diluted extracts. Serum and kidney IGF-I levels were measured by RIA using a polyclonal rabbit antibody (Nichols Institute Diagnostics, San Capistrano, CA, USA) and recombinant human IGF-I as the standard (Amersham International). The kidney tissue IGF-I concentrations were corrected for the contribution of entrapped serum IGF-I [18, 19]. Monoiodinated IGF-I [^{125}I-(Tyr^{31})-IGF-I] was obtained from Novo-Nordisk A/S (Bagsværd, Denmark). The intra-assay and interassay coefficients of variations for both assays were less than 5% and 10% for both assays.

Western ligand blotting for determination of serum insulin-like growth factor-binding protein 3

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western ligand blotting (WLB) were performed according to the method of Hossenlopp et al [21] as previously described [22]. Two microliters of serum were subjected to SDS-PAGE (10% polyacrylamide) under nonreducing conditions. The electrophoresed proteins were transferred by electroelution onto nitrocellulose paper (Schleicher & Schuell, Munich, Germany), and membranes were incubated overnight at 4°C with approximately 500,000 cpm ^{125}I-IGF-I (specific activity 2000 Ci/mmol) in 10 ml 10 mmol/liter Tris-HCl buffer (TBS) containing 1% bovine serum albumin and 0.1% Tween (pH 7.4). Membranes were washed with TBS, and after drying overnight, the nitrocellulose sheets were autoradiographed with Kodak X-AR film and exposed to DuPont-New England Nuclear (Boston, MA, USA) enhancing screens at −80°C for three to seven days. Specificity of the IGFBP bands was ensured by competitive coincubation with unlabeled IGF-I purchased from Bachem (Bubendorf, Switzerland). On WLB (with ^{125}I-IGF-I as ligand), IGFBP-3 appeared as a 38 to 42 kDa doublet band corresponding to the intact acid-stable IGF-binding subunit of IGFBP-3.

Northern blotting for determination of kidney GHR, GHBP, and IGF-I mRNA

Renal gene expression of GHR, GHBP, and IGF-I was measured by Northern blot analysis. Total RNA was extracted from kidney samples by the guanidinium thiocyanate method as previously described [23]. Glyoxilated RNA samples were electrophoresed in 1% agarose gels submerged in 10 mM sodium phosphate, pH 7.2, and transferred to nylon membranes (Hybond N$^+$; Amer-

sham, Hertogenbosch, The Netherlands). Filters were hybridized with 1 to 2 10^6 cpm/ml of ^{32}P-labeled cDNA fragments encoding for GHR/GHBP [24], IGF-I (kindly provided by Dr. G.I. Bell, Chicago, IL, USA), and 18S rRNA at 65°C according to the method of Church and Gilbert [25].

Quantitation of gels

Western ligand blots were quantitated by densitometry using a Shimadzu CS-9001 PC dual wavelength flying spot scanner (Shimadzu Europe GmbH, Duisburg, Germany), and the relative densities were expressed as pixel density. Northern blots were scanned on a Phosphor Imager (Molecular Dynamics, Sunnyvale, CA, USA) and quantitated using ImageQuant software. All measured mRNA results were expressed relative to 18S rRNA levels as arbitrary units.

Estimation of glomerular volume

A 2 mm thick horizontally cut slice from the middle of the right kidney (containing the papilla) was fixed in 4% paraformaldehyde and embedded in Technovit. Two micron thick sections were cut on a rotation microtome and stained with periodic acid-Schiff stain and hematoxylin. The mean glomerular tuft volume (V_G) was determined from the mean glomerular cross-sectional area (A_G) by light microscopy, as previously described [26, 27]. Profile areas were traced using a computer-assisted morphometric unit (Image Tool; The University of Texas Health Science Center, San Antonio, TX, USA). A_G was determined as the average area of a total of 40 to 80 glomeruli (tuft omitting the proximal tubular tissue within the Bowmann capsule) and V_G calculated as follows:

$$V_G = \beta/k \times (A_G)^{3/2}$$

where $\beta = 1.38$ is the shape coefficient for spheres (the idealized shape of glomeruli), and $k = 1.1$ is a size distribution coefficient [26, 27].

Statistics

Analysis of variance analyses (ANOVA) for repeated measurement were used to evaluate differences in combination with Student's *t*-test for unpaired comparisons. A P value of less than 0.05 was regarded as significant. Means are given ± SEM.

RESULTS

Body weight, food consumption, and blood glucose

Both groups of animals exposed to unilateral nephrectomy (placebo and G120K-PEG treated) had a minor decrease (8 to 11%) in body weight two days after operation ($P < 0.05$), whereas no differences were seen on day 7 (Fig. 1A). Similarly, food consumption (expressed as g/24 hr) decreased transiently in both uninephrectomized groups at day 2 (12%, $P < 0.05$) when compared with sham-operated animals, whereas no difference was seen on day 7 (Fig. 1B). Again, G120K-PEG treatment did not affect the food intake in either group (Fig. 1B). No changes in blood glucose levels were seen in any group at any time point (data not shown).

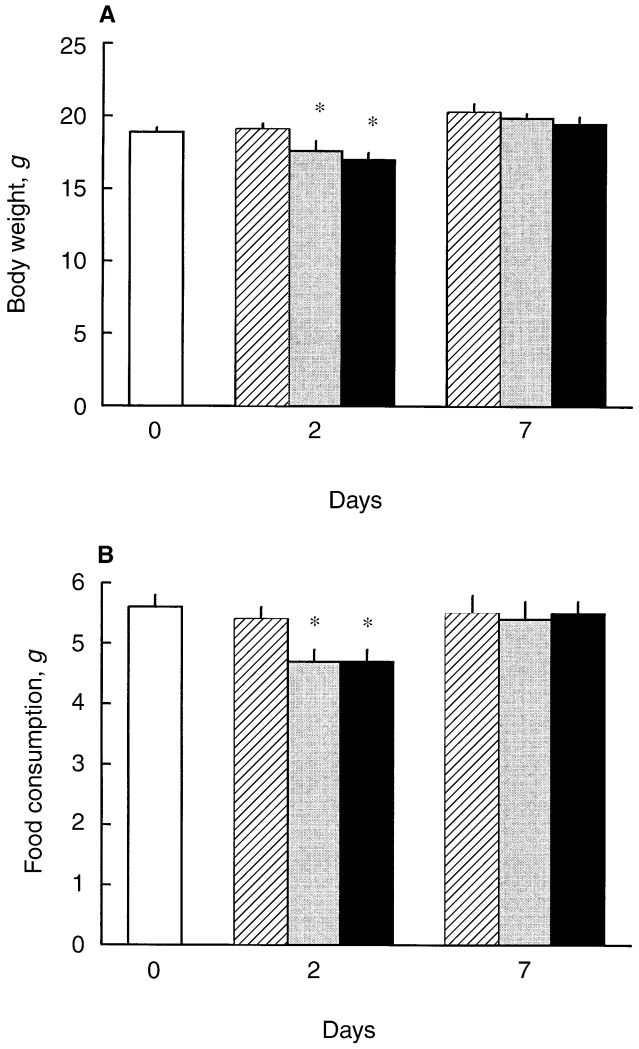

Fig. 1. Changes over seven days in body weight (*A*) and food consumption (*B*) in nonoperated controls (□), sham-operated animals (▨), uninephrectomized animals treated with placebo (▩), or G120K-PEG (2 mg/kg/body wt every second day; ■). Values are mean ± SEM (N = 6 in each group). *$P < 0.05$ vs. sham-operated group on day 2.

Serum GH, IGF-I, IGFBP-3, and kidney IGF-I

Figure 2 shows serum GH, IGF-I, and IGFBP-3 levels in the four experimental groups over the study period of seven days. It has been shown previously that barbital anesthesia induces a rise in GH levels lasting for up to 90 minutes [28], and accordingly, the endogenous GH

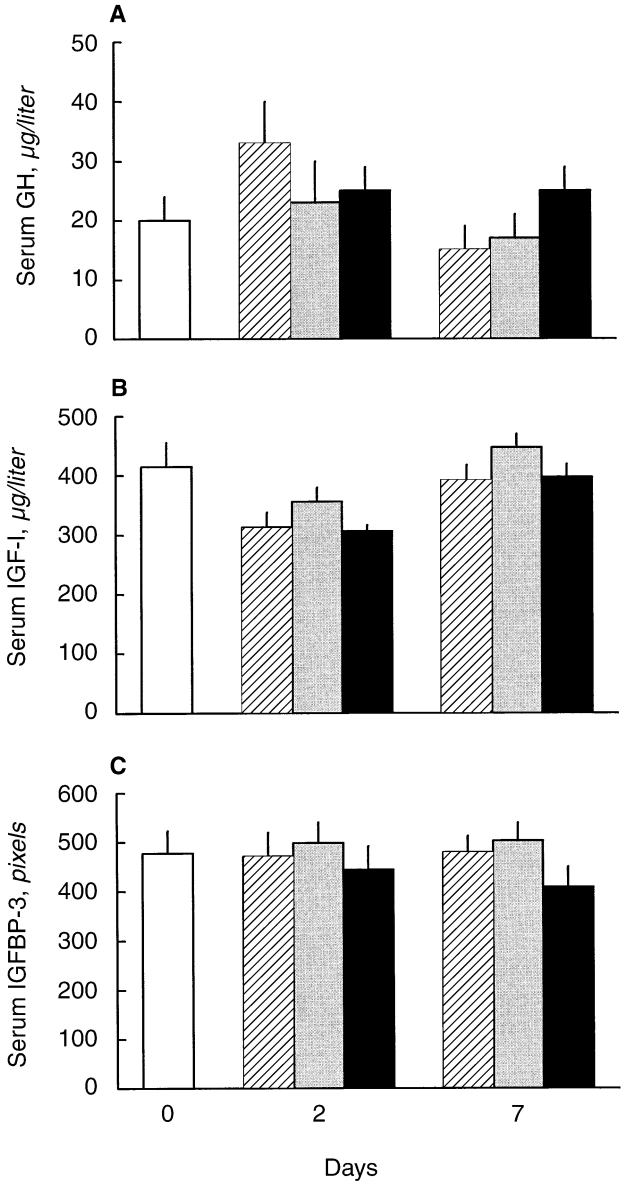

Fig. 2. Mean serum growth hormone (GH; *A*), insulin-like growth factor I (IGF-I; *B*), and IGF binding protein 3 (IGFBP-3; *C*) levels in nonoperated controls (□), sham-operated animals (▨), uninephrectomized animals treated with placebo (▩), or G120K-PEG (■). Values are mean ± SEM ($N = 6$ in each group).

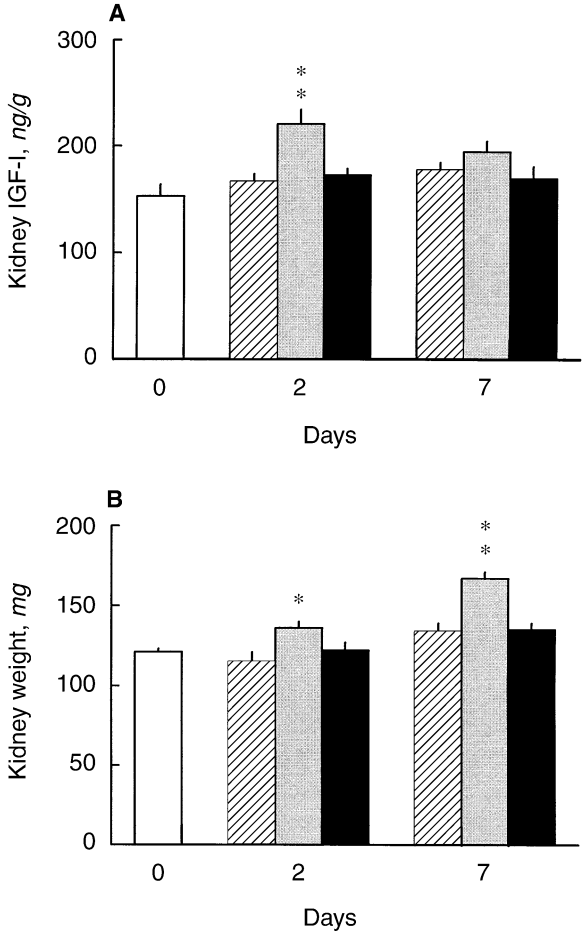

Fig. 3. Mean kidney insulin-like growth factor I (IGF-I) levels (*A*) and kidney weight (*B*) over seven days in nonoperated controls (□), sham-operated animals (▨), uninephrectomized animals treated with placebo (▩), or G120K-PEG (■). Values are mean ± SEM ($N = 6$ in each group). *$P < 0.05$; **$P < 0.01$ vs. all other groups.

levels given in Figure 2A are stimulated values. No differences in serum GH levels were seen in response to uninephrectomy *per se*, and furthermore, no effect of G120K-PEG administration was seen (Fig. 2A). In addition, serum IGF-I and IGFBP-3 were unchanged in all groups over time with no effect of G120K-PEG treatment (Fig. 2 B, C). Kidney IGF-I increased transiently in placebo-treated uninephrectomized animals on day 2 (32%, $P < 0.05$) when compared with sham-operated controls, with a decrease on day 7 to a level not significantly different from controls (Fig. 3A). The increase in kidney IGF-I was fully abolished in G120K-PEG–treated nephrectomized animals (Fig. 3A).

Kidney weight, glomerular volume, and liver weight

The kidney weight increase in the untreated uninephrectomized group over the study period amounted to 28% after seven days when compared with sham-operated controls ($P < 0.01$). The kidney weight increase was fully abolished in the G120K-PEG–treated uninephrectomized animals (Fig. 3B). The increase in glomerular volume in untreated uninephrectomized animals amounted to 42% at day 7 when compared with sham-operated controls ($P < 0.01$), whereas the glomerular volume of G120K-PEG–treated uninephrectomized mice was fully abolished with a value comparable with the two control groups (Fig. 4). No difference in liver weight was found on day 7 between sham-operated, placebo-treated, or G120K-PEG–treated uninephrectomized animals (S, 1130 ± 46 mg; NP, 1131 ± 36 mg; NA, 1150 ± 51 mg).

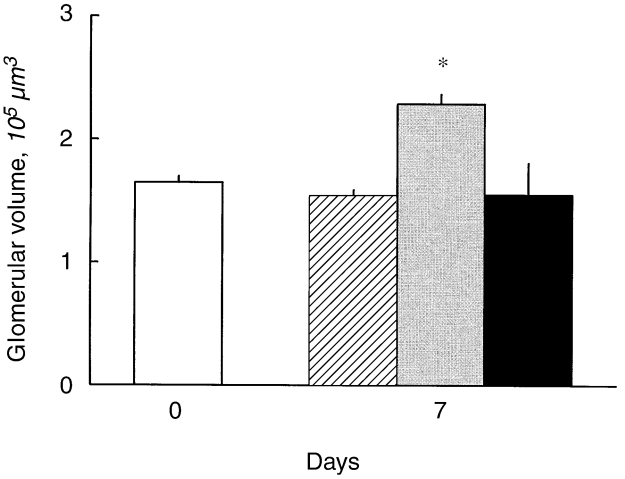

Fig. 4. Mean glomerular volume on day 0 in nonoperated controls (□) and on day 7 in sham-operated animals (▨), uninephrectomized animals treated with placebo (▩) or G120K-PEG (■). Values are mean ± SEM ($N = 6$ in each group). *$P < 0.05$ vs. all other groups.

Kidney GHR, GHBP, and IGF-I mRNAs

Figure 5 shows the renal expression of GHR, GHBP, and IGF-I over the study period of seven days. GHR/GHBP mRNA levels were unchanged over time in sham-operated animals, whereas a decrease was observed in the untreated uninephrectomized group at day 7 ($P < 0.01$). No decrease in GHR/GHBP mRNA levels was observed in G120K-PEG–treated uninephrectomized animals (Fig. 5). Kidney IGF-I mRNA decreased in untreated uninephrectomized mice at day 7 ($P < 0.01$), whereas in G120K-PEG–treated uninephrectomized animals, it decreased both at days 2 and 7 (both $P < 0.01$; Fig. 5).

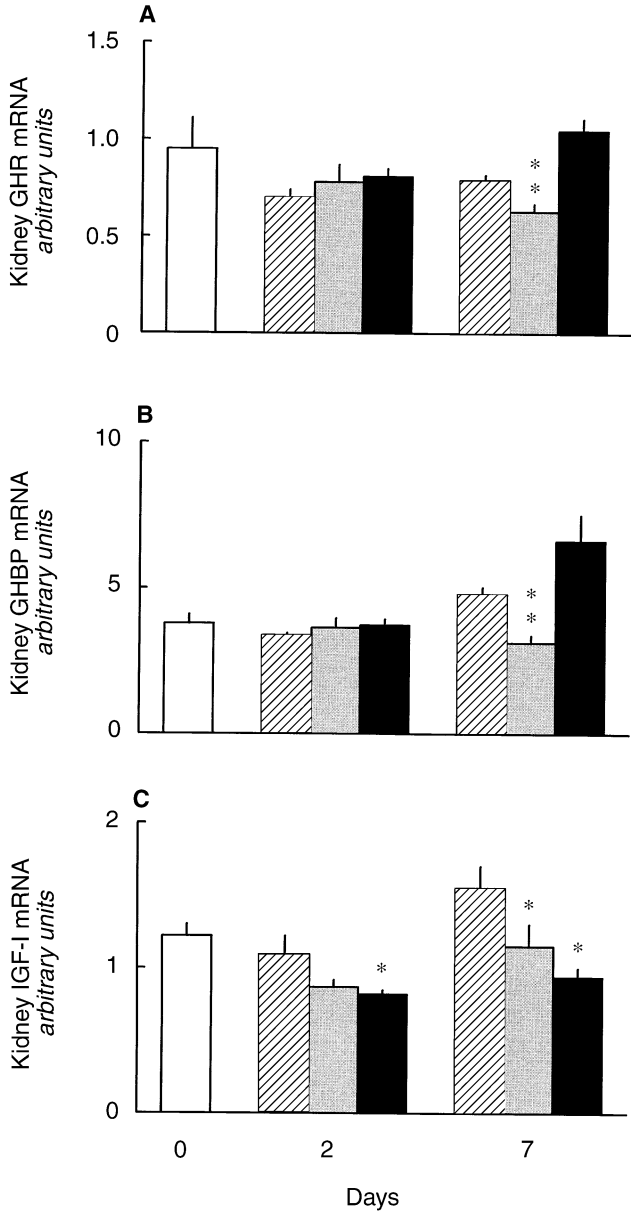

Fig. 5. Mean kidney growth hormone receptor (GHR) mRNA (*A*), GH-binding protein (GHBP) mRNA (*B*), and insulin-like growth factor I (IGF-I) (*C*) mRNA levels over seven days in nonoperated controls (□), sham-operated animals (▨), uninephrectomized animals treated with placebo (▩), or G120K-PEG (■). Values are mean ± SEM ($N = 6$ in each group). **$P < 0.01$ vs. other groups; *$P < 0.01$ vs. sham-operated group.

DISCUSSION

The major new finding of this study is an inhibitory effect on CRG of an exogenously administered specific GHR antagonist in adult uninephrectomized mice, thereby indicating a strictly permissive role of GH in CRG.

For decades, GH and IGFs have been suggested as pathogenic factors in CRG following unilateral nephrectomy [1–10]. The first indirect evidence for this hypothesis was presented in the 1970s by the observation that the CRG response was blunted in uninephrectomized, hypophysectomized rats [1, 2]. In addition, adult rats exposed to unilateral nephrectomy have been shown to exert increased GH pulsatility [10], and furthermore, the administration of a long-acting somatostatin analogue (octreotide) [6] or a GH-releasing factor antagonist [9] has been shown to abolish the early CRG. Despite the mentioned evidence for a pathogenic role of GH in CRG, so far the exact role of GH, as a modulating or a strictly permissive factor for CRG in adult rodents, has not yet been resolved. By recognizing the potential role that GH and IGFs may play in various pathophysiological conditions (for example, diabetic kidney disease [17–20]), a series of highly specific antagonists of the GH action has been recently developed. The discovery of these new GHR antagonists enabled us to perform a study in which the direct role of GH in CRG could be answered. Initially, when the GHR antagonists were discovered, it was shown that an alteration of single

amino acids in the third α-helix of bovine (b) GH (residues 109 to 126) resulted in a GH antagonist [12–14, 16]. *In vitro* experiments showed that the group of GH antagonists binds to the GHR with the same affinity as native GH, but *in vivo*, a phenotypic dwarf animal characterized by low circulating IGF-I levels and a proportional body composition develops when the GH antagonist is expressed in transgenic mice [15, 16]. Accordingly, studies were published describing renoprotective effects of GH antagonists in long-term diabetic transgenic mice that express GH antagonists (bGH-G119R and hGH-G120R) [17]. Recently, we showed that the administration of a pegylated GHR antagonist (G120K-PEG) in experimental diabetes in mice abolished renal IGF-I accumulation, renal enlargement, and glomerular hypertrophy and diminished the increase in urinary albumin excretion through a specific mechanism independent of changes in body weight, food consumption, metabolic control, serum GH, IGF-I, or IGFBP-3 [18].

Along with this observation we demonstrate, in this study, inhibition of CRG and glomerular hypertrophy in uninephrectomized mice treated with G120K-PEG for one week. The animals received G120K-PEG subcutaneously every second day to maintain high diurnal levels [18]. No toxic effects of G120K-PEG treatment were observed, as treated mice had similar changes in body weight and food consumption when compared with their placebo-treated group. As observed in the diabetes study mentioned earlier in this article [18], the renal effects of GHR antagonism were observed without detectable changes in circulating GH, IGF-I, or IGFBP-3 levels, indicating a specific effect of G120K-PEG through a blockade of the renal GHR. This is further supported by the fact that the increase in kidney IGF-I observed in untreated diabetic animals was fully abolished in G120K-PEG–treated animals. Finally, the weight of a reference organ (that is, the liver) was unaffected by GHR antagonist treatment.

As stated earlier in this article, unilateral nephrectomy in adult rats has been characterized by increased GH-pulsatility [10]. However, in this study, we were unable to demonstrate changes in barbital stimulated GH levels in uninephrectomized mice after two and seven days when compared with nonoperated and sham-operated controls. These apparent different findings are easily explained by the fact that the previously mentioned GH hyperpulsatility was mainly detectable 24 hours after uninephrectomy with decreasing levels after 48 hours [10]. Furthermore, multiple sequential measurements of GH pulsatility, as performed previously [10], are better estimates of pertubations in circulating GH than a single GH measurement (albeit barbital stimulated), as performed in this study.

The transient increase in kidney IGF-I observed in this study is in concert with previous results reported in adult rats from different laboratories [3, 5–7]. In this study, the administration of G120K-PEG completely prevented the obligatory early rise in kidney IGF-I along with the morphological changes (that is, renal size and glomerular volume). This observation is similar to the effect seen during octreotide administration (a long-acting somatostatin analogue) in CRG [6]. Although the transient rise in renal IGF-I peptide seems to be a well-recognized phenomenon [3, 5–7], the molecular basis for the increase is still a matter of dispute [7–10]. A recent study reported unchanged renal GHR mRNA levels in immature and adult rats in response to uninephrectomy [11]. Although immature rats exposed to unilateral nephrectomy have increased levels of IGF-I, IGF-I receptor (IGF-IR), and IGF-II/mannose-6-phosphate receptor (IGF-II/Man-6-PR) mRNA [8], IGF-I mRNA has been reported to be unchanged [7] or decreased [4, 8, 11] along with decreased levels of IGF-IR and IGF-II/Man-6-PR mRNA in uninephrectomized adult rats [8, 11]. In this study, a decrease in renal GHR/GHBP mRNA was seen in untreated uninephrectomized animals at day 7 when the renal growth rate had abated. In contrast, no decrease in renal GHR/GHBP mRNA was observed in G120K-PEG–treated animals, which most likely reflects a compensatory response at mRNA level to the functional GHR blockade induced by the GHR antagonist. In concert with previous studies, kidney IGF-I mRNA was decreased in nephrectomized animals at day 7, whereas G120K-PEG treatment induced a modest, but still significant, decrease in IGF-I mRNA at day 2, along with inhibition of the early rise in kidney IGF-I peptide.

In conclusion, the administration of a GHR antagonist in uninephrectomized adult mice has an inhibitory effect on increased kidney IGF-I, CRG, and glomerular hypertrophy, without affecting circulating levels of GH, IGF-I, or IGFBP-3. Accordingly, the mechanism of the renal effects of the GHR antagonist may be mediated through an inhibitory effect on renal IGF-I accumulation through the renal GHR. This study demonstrates the central role of the GH/IGF axis in CRG and shows, to our knowledge for the first time, the strictly permissive role of GH in the process in adult mice.

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Reprint requests to Allan Flyvbjerg, M.D., D.Sc., Medical Department M/Medical Research Laboratory M, Institute of Experimental Clinical Research, University Hospital, Kommunehospitalet, DK-8000 Aarhus C, Denmark.
E-mail: allan.flyvbjerg@dadlnet.dk

REFERENCES

1. Ross J, Goldman JK: Compensatory renal hypertrophy in hypophysectomized rats. *Endocrinology* 87:620–627, 1970
2. Dicker SE, Greenbaum AL, Morris CA: Compensatory renal hypertrophy in hypophysectomized rats. *Am J Physiol* 273:241–253, 1977
3. Stiles AD, Sosenko IR, D'Ercole AJ, Smith BT: Relation of kidney tissue somatomedin C/insulin-like growth factor I to postnephrectomy renal growth in the rat. *Endocrinology* 117:2397–2401, 1985
4. Fagin JA, Melmed S: Relative increase in insulin-like growth factor I messenger ribonucleic acid levels in compensatory renal hypertrophy. *Endocrinology* 120:718–724, 1987
5. Flyvbjerg A, Thorlacius-Ussing O, Næraa R, Ingerslev J, Ørskov H: Kidney tissue somatomedin C and initial renal growth in diabetic and uninephrectomized rats. *Diabetologia* 31:310–314, 1988
6. Flyvbjerg A, Frystyk J, Thorlacius-Ussing O, Ørskov H: Somatostatin analogue administration prevents increase in kidney somatomedin C and initial renal growth in diabetic and uninephrectomized rats. *Diabetologia* 32:261–265, 1989
7. Lajara R, Rotwein P, Bortz JD, Hansen VA, Sadow JL, Betts CR, Rogers SA, Hammerman MR: Dual regulation of insulin-like growth factor I expression during renal hypertrophy. *Am J Physiol* 257:F252–F261, 1989
8. Molroney SE, Haramati A, Werner H, Bondy C, Roberts CT Jr, Leroith D: Altered expression of insulin-like growth factor I (IGF-I) and IGF receptor genes after unilateral nephrectomy in immature rats. *Endocrinology* 130:249–256, 1992
9. Molroney SE, Lumpkin MD, Roberts CT Jr, Leroith D, Haramati A: Effect of growth hormone-releasing factor antagonist on compensatory renal growth, insulin-like growth factor I (IGF-I) and IGF receptor genes after unilateral nephrectomy in immature rats. *Endocrinology* 130:2697–2702, 1992
10. Haramati A, Lumpkin MD, Molroney SE: Early increase in pulsatile growth hormone release after unilateral nephrectomy in adult rats. *Am J Physiol* 266:F628–F632, 1994
11. Fervenza FC, Tsao T, Hsu F, Rabkin R: Intrarenal insulin-like growth factor I axis after unilateral nephrectomy in rat. *J Am Soc Nephrol* 10:43–50, 1999
12. Chen WY, Wight DC, Mehta BV, Wagner TE, Kopchick JJ: Glycine 119 of bovine growth hormone is critical for growth-promoting activity. *Mol Endocrinol* 5:1845–1852, 1991
13. Fuh G, Cunningham BC, Fukunaga R, Nagata S, Goeddel DV, Wells JA: Rational design of potent antagonists to the human growth hormone receptor. *Science* 256:1677–1680, 1992
14. Ross C, Olsen K, Fuh G, Marian M, Mortensen D, Teshima G, Chang S, Chu H, Mukku V, Canova-Davis E, Somers T, Cronin M, Winkler M, Wells JA: Long-acting growth hormones produced by conjugation with polyethylene glycol. *J Biol Chem* 271:21969–21977, 1996
15. Chen WY, Wight DC, Wagner TE, Kopchick JJ: Expression of a mutated bovine growth hormone gene suppresses growth of transgenic mice. *Proc Natl Acad Sci USA* 87:5061–5065, 1990
16. Chen WY, White ME, Wagner TE, Kopchick JJ: Functional antagonism between endogenous mouse growth hormone (GH) and a GH analog results in dwarf transgenic mice. *Endocrinology* 129:1402–1408, 1991
17. Chen N-Y, Chen WY, Kopchick JJ: A growth hormone antagonist protects mice against streptozotocin induced glomerulosclerosis even in the presence of elevated levels of glucose and glycated hemoglobin. *Endocrinology* 137:5163–5165, 1996
18. Flyvbjerg A, Bennett WF, Rasch R, Kopchick JJ, Scarlett JA: Inhibitory effect of a growth hormone receptor antagonist (G120K-PEG) on renal enlargement, glomerular hypertrophy, and urinary albumin excretion in experimental diabetes in mice. *Diabetes* 48:377–382, 1999
19. Flyvbjerg A, Frystyk J, Østerby R, Ørskov H: Kidney IGF-I and renal hypertrophy in GH deficient dwarf rats. *Am J Physiol* 262:E956–E962, 1992
20. Flyvbjerg A: Role of growth hormone, insulin-like growth factors (IGFs) and IGF-binding proteins in the renal complications of diabetes. *Kidney Int* 52(Suppl 60):S12–S19, 1997
21. Hossenlopp P, Seurin D, Segovia-Quinson B, Hardouin S, Binoux M: Analysis of serum insulin-like growth factor binding proteins using Western blotting: Use of the method for titration of the binding proteins and competitive binding studies. *Anal Biochem* 154:138–143, 1986
22. Flyvbjerg A, Kessler U, Dorka B, Funk B, Ørskov H, Kiess W: Transient increase in renal insulin-like growth factor binding proteins during initial kidney hypertrophy in experimental diabetes in rats. *Diabetologia* 35:589–593, 1992
23. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-cloroform extraction. *Anal Biochem* 162:156–159, 1987
24. Mathews LS, Enberg B, Norstedt G: Regulation of rat growth hormone receptor gene expression. *J Biol Chem* 264:9905–9910, 1989
25. Church GM, Gilbert W: Genomic sequencing. *Proc Natl Acad Sci USA* 81:1991–1995, 1984
26. Weibel ER: *Stereological Methods: Practical Methods for Biological Morphometry.* London, Academic Publishers, 1979, pp 51–57
27. Pagtalunan ME, Rasch R, Rennke HG, Meyer TW: Morphometric analysis of effects of angiotensin II on glomerular structure in rats. *Am J Physiol* 268:F82–F88, 1995
28. Takahashi K, Daughaday WH, Kipnis DM: Regulation of immunoreactive growth hormone secretion in male rats. *Endocrinology* 88:909–917, 1971